

REPORTS
TO THE
LOCAL GOVERNMENT BOARD
ON
PUBLIC HEALTH AND MEDICAL
SUBJECTS.

(NEW SERIES, No. 103.)

Report to the Local Government Board by
Dr. G. W. Monier-Williams on the Freezing
Point of Milk considered in its relation to
the detection of added water.

[*Food Reports*, No. 22.]



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CONTENTS.

	PAGE
I.—Previous investigations 	1
II.—Precautions to be observed in determining freezing points ...	3
III.—Description of apparatus and method employed 	7
The freezing points of cane sugar solutions of known strengths 	10
Determination of the super-cooling correction (K) for milk	12
IV.—Results obtained with milk samples from different sources ...	12
V.—Discussion of results 	18
Simplification of experimental procedure in freezing point determinations 	22
Effect of development of acidity in the milk 	22
Effect of removal of the fat 	23
Effect of sterilisation by heat 	24
Calculation of the amount of added water present 	26
VI.—The osmotic pressure of milk and the ratio of lactose to chlorine	27
VII.—Conclusions 	29

Report to the Local Government Board by
Dr. G. W. Monier-Williams on the Freezing
Point of Milk considered in its relation
to the detection of added water.

ARTHUR NEWSHOLME,

Medical Officer,

January 11th, 1915.

During the last fifteen or twenty years a large number of papers have been published, mainly by continental chemists, calling attention to the fact that the freezing point of milk varies within comparatively narrow limits. The addition of water to the milk has the effect of raising the freezing point (*i.e.*, of reducing the depression of the freezing point with reference to that of water) in proportion to the quantity of water added, so that if a milk of original freezing-point -0.540° is adulterated so as to contain 10, 25, or 50 per cent. of added water, the freezing points of the resulting mixtures would be -0.486° , -0.405° and -0.270° respectively. It is claimed that the value observed for the freezing point affords a more certain and accurate indication of the presence and relative amount of added water in milk than can be obtained from the results of chemical analysis alone.

The value of such a method as applied to the analysis of milk samples will depend in the first place upon the extent to which the freezing point of genuine milk may be subject to natural variation, and in the second place upon the rapidity and degree of accuracy with which the determination can be carried out.

I. PREVIOUS INVESTIGATIONS.

The statements published by various investigators as to the limits of natural variation in the case of pure milk are somewhat divergent. Owing to the apparent omission in the majority of cases of certain precautions which are essential to an accurate determination of the freezing point, it is difficult to say how far these divergent results are due to natural causes, or to errors of experiment.

The earliest paper dealing with this subject was published by Dreser* in 1892. He compared the osmotic pressure of blood serum and other biological sera by means of freezing point determinations, and found that the freezing point of cows' milk varied between -0.55° and -0.57° , while that of the blood serum of cows was -0.58° to -0.59° . Two years later Beckmann and Jordis pointed out that the method could be applied to the detection of added water in milk, and during the ensuing decade a large

* On page 30 *et seq.* are given references to the papers referred to in the text.

number of papers appeared, some of them dealing with the osmotic pressure of blood serum from a purely physiological point of view, and others with the freezing point of milk and the detection of added water. Hamburger, Bugarszky and Tangl, von Korányi, Koeppe, Strauss, Achard, Nagelschmidt and others, dealt chiefly with the freezing point of blood serum, and their work confirmed in the main the earlier observations of Dreser as to its approximate constancy for a particular species. Koeppe, Nagelschmidt and Strauss showed moreover that artificial alteration in the molecular concentration of the blood was followed by a similar alteration in the osmotic pressure of the milk.

Winter, who appears to have published a number of papers on this subject between the years 1895 and 1904, was led to the conclusion that the osmotic pressures of blood serum and milk are intimately connected and practically constant. He held that the freezing point of the milk from single cows never falls outside the limits of -0.54° and -0.57° , and that the mixed milk of a herd is practically constant at -0.55° to -0.56° .

Winter's statements were controverted by Bordas and Génin, who investigated 50 samples of milk from single cows, and recorded freezing points varying from -0.440° to -0.560° . In a subsequent series of experiments, in which more attention was paid to possible sources of error, and the necessary corrections were introduced, the freezing point was found to vary from -0.512° to -0.529° . It will be seen that Bordas and Génin's results, although differing to a considerable extent from those of Winter, indicate a somewhat remarkable degree of constancy in the freezing point of milk. Objections were urged against these results on the ground that sufficient care was not taken to ensure the absolute genuineness of the milk samples in question, but in view of the results recorded in the present report it would appear probable that Bordas and Génin's work is as accurate as any that has hitherto been published on the subject, and that the high values recorded by the majority of other observers are due to experimental errors.

Results very similar to those of Winter were obtained by Hamburger, van der Laan, Carlinfanti, Koeppe, Hotz, Schnorf, Barthe, Bertozzi, Pins, Atkins and many others. Hamburger found slight differences in freezing point between "fore" milk and "stippings," but this was not confirmed by subsequent observers. Hamburger, Jacoangeli and Pins stated that evening milk shows a slightly higher value for Δ^* than morning milk, but

* Some confusion is likely to arise from the terms "higher" and "lower" as applied to freezing points lying below zero. A freezing point of -0.520° is obviously higher than one of -0.550° , but it is apparently the usual custom among writers on the subject to express the results obtained in terms of the *depression* of the freezing point with reference to water, generally expressed by the symbol Δ . Thus a value of 0.550° would be higher than one of 0.520° , as representing a greater depression of the freezing point. The adoption of the latter mode of expression has much to recommend it and tends to avoid confusion as the terms higher and lower correspond with the actual figures and the constant repetition of the minus sign is avoided. In the present report therefore the symbol Δ is used throughout to indicate the difference between the freezing point of milk and that of water.

Maiocco appears to have come to the opposite conclusion, and Abati and Sohn could find no regular difference to exist between morning and evening milk.

The freezing point has been shown to be independent of differences in breed, age or period of lactation. The majority of observers seem to be in agreement that the removal of fat from milk has no effect upon the figure obtained, but here again there are several conflicting statements.

Sterilisation in closed vessels is stated to be without influence upon the freezing point, but Hotz found that the value for Δ is raised by boiling in open vessels owing to the increased concentration consequent on the escape of water vapour.

The formation of lactic acid during souring has, on the other hand, a very great influence on the value obtained, owing to the increase in the total number of molecules in solution. Stoecklin, who has applied the method to several thousand milk samples in France, claims that the original freezing point of the fresh milk may be calculated from that of the milk after souring, by taking into account the degree of acidity developed.

Henderson and Meston state that the freezing point of the mixed milk of herds in Southern Queensland is practically constant at -0.55° to -0.56° with a mean of -0.555° , and that the percentage of added water may be determined by this method with a high degree of accuracy.

In view of the fact that this method has been used in the routine examination of milk samples for a considerable number of years in several continental laboratories, and has apparently been found satisfactory, it is somewhat remarkable that, with the exception of a short paper by Atkins, no detailed investigation of it has been made in this country. If the statements which have been made concerning it could be confirmed, it would undoubtedly constitute a valuable addition to the existing methods of milk control.

It appeared, therefore, desirable that an investigation into the freezing points of a considerable number of genuine milk samples should be undertaken with all precautions necessary to ensure a high degree of accuracy, with a view to ascertaining whether the method is capable of being used with advantage in the control of the milk supply, and whether the indications afforded by it are more accurate than the well-known chemical methods already in use. The question derives additional interest from the fact that in Queensland, according to Regulations made under the Health Acts 1900-1911, genuine milk is required to show a freezing point not higher than -0.55° below zero (*i.e.*, a freezing point depression (Δ) not less than 0.55°).

II. PRECAUTIONS TO BE OBSERVED IN DETERMINING FREEZING POINTS.

In order to determine the freezing point of an aqueous solution with a degree of accuracy approaching $\pm 0.001^{\circ}$, it is necessary to take into consideration a number of possible sources of error, some of which may influence the determination to a very large extent.

Of the sources of error inherent in a mercury thermometer the most important are :—

- i. Inaccuracy of the scale.
- ii. Slight alterations in the volume of the bulb, by which the zero point may be either raised or lowered. Most thermometers undergo a gradual contraction of the bulb which may continue for several months and necessitate repeated corrections of the zero point. Sudden alterations may also occur for no apparent reason, and variations in the barometric pressure may cause the zero point to rise or fall several thousandths of a degree.
- iii. The large heat capacity of the bulb in thermometers with a very open scale. This may cause an appreciable time to elapse before the correct temperature is registered.
- iv. The resistance offered to the movement of the mercury in a very fine capillary, which results in "lag."
- v. The temperature of the emergent column of mercury.

The thermometer used in all the following experiments had a total range of about one degree, from -1° to $+0.2^{\circ}$, the divisions being engraved upon the stem and corresponding to intervals of 0.005° . Each 0.005° division occupied a length of approximately 0.4 millimetres, so that temperature differences of 0.0005° could be estimated by means of a lens or telescope. The bulb was of Jena normal glass, and measured approximately 50 mm. in length by 8 mm. external diameter. The fine capillary of the stem was of circular cross section.

The calibration of a thermometer of this kind is an exceedingly difficult matter, and for the purpose of these experiments it appeared that the most reliable method of controlling the accuracy of the scale was by means of a careful comparison of the freezing points of cane sugar solutions of known composition with the very accurate values given by Raoult, due regard being paid to all possible sources of error. As will be seen from the table on page 11, the greatest difference observed between the freezing points thus found and those calculated from an equation given by Raoult was $\pm 0.0025^{\circ}$. As this difference is of the same order as the probable experimental error due to other factors, it is unlikely that increased accuracy could have been secured by more elaborate methods of calibration.

The second source of error referred to above may be practically eliminated by taking the freezing point of distilled water both before and after each daily series of determinations. The zero point of the thermometer used in these experiments, when determined in this way, varied from time to time between $+0.016^{\circ}$ and $+0.024^{\circ}$ on the scale, but the variation on any one day was never more than 0.003° , and in most cases no difference at all was observed between the two readings. When such variation between morning and evening determinations was observed, the mean of the two values was taken as the zero point. Any error due to the large heat capacity of the bulb is eliminated if the freezing point of the liquid coincides with the "temperature of convergence" (*see below*).

The "lag" or sticking of the mercury in the fine capillary may be overcome by the use of a small electric hammer which gives a succession of sharp taps to the thermometer. It was found in these experiments that the freezing point registered by the

thermometer was almost always about 0.005° too low unless efficient vibrations were set up in the thermometer to counteract this tendency of the mercury to stick in the capillary. Repeated observations of the freezing point of distilled water showed that these vibrations did not cause any sudden alteration of the zero point during the course of a day's determinations.

In certain cases the error due to the mercury of the emergent column being at a higher temperature than that of the bulb may be considerable. If the temperature of the room does not vary during the time occupied in the determinations of the freezing points of water and of the solution, the necessary correction will apply only to the length of the column corresponding to the difference between the two freezing points, and may easily be calculated. For a difference of 0.5° , as is approximately the case with milk, and a room temperature of 20° , this correction will be -0.0015° . If, however, the room temperature varies, the total length of the emergent column above the liquid must be taken into account. In the ordinary Beckmann thermometer, owing to its peculiar construction, it is not easy to arrive at the total length of this emergent column, which may be as much as six or seven degrees. A variation in the room temperature of 3° between two experiments would introduce under these circumstances an additional error of 0.003° . In order to eliminate as far as possible this latter source of error, the thermometer used in these experiments was made with a very short stem, the graduation mark corresponding to -1° , being only 38 mm. above the top of the bulb. Since the graduation marks below -0.7° were obscured by the cork stopper of the freezing vessel, an additional thermometer, not shown in the illustration on page 8, graduated into 0.1° , was inserted in order to ascertain the extent to which the solutions were super-cooled.

The most important sources of error apart from those inherent in the thermometer are the following:—

- i. Mechanical production of heat by the stirrer.
- ii. Too low a temperature of the freezing bath.
- iii. Increase in the concentration of the solution owing to separation of ice (super-cooling error).

The first two of these errors are eliminated if the temperature of the bath is so regulated that the freezing point of the solution coincides with the so-called "temperature of convergence." The temperature of convergence is that temperature at which the solution is in exact heat equilibrium with its surroundings, the amount of heat abstracted from the solution in unit of time by the freezing bath being exactly equal to the heat imparted by the stirrer, by radiation from the outside, etc. It can be shown mathematically (Raoult), that only under these conditions does the apparent freezing point of the solution coincide with its true freezing point.

The convergence temperature is easily ascertained by actual trial (Raoult), and will vary with each apparatus and method of working. In the apparatus described on page 8 it was 0.24° above the bath temperature, with the stirrer rotating at 1,300 revolutions per minute. Therefore, if the freezing point of the

solution is approximately -0.50° , the temperature of the bath must be -0.74° in order that the solution at its freezing point shall be in exact heat equilibrium with its surroundings. If the bath is kept at a very much lower temperature, as is usually the case with mixtures of ice and salt, the apparent freezing point will be lower than the true value (*i.e.* the depression (Δ) will be greater), and on the other hand, if the heat imparted by stirring be increased, too high a freezing point will be observed. In this apparatus it is an easy matter to regulate the temperature of the bath so that at the moment of freezing the temperature of convergence shall coincide with the freezing point. Extreme accuracy in the regulation of the bath temperature is not necessary, a variation of $+0.02^{\circ}$ being without influence on the result. A further advantage in this manner of working is that the temperature of the solution at its freezing point remains constant for an indefinite length of time, thus facilitating the taking of accurate readings, and eliminating any error due to the large heat capacity of the bulb.

As a matter of fact the difference between the apparent and the true freezing point of a solution, when the convergence temperature is lower than the freezing point, is comparatively small. With distilled water, however, in the determination of the zero point, an unduly low bath temperature may give rise to far more serious errors. A thin film of ice is very liable to be formed on the inner surface of the freezing vessel. Where such an ice film has been formed, the effect of alterations in the temperature of the freezing mixture is merely to increase or diminish the thickness of the film, without any influence being exerted upon the bulk of the water in the freezing vessel. Heat is imparted by the stirrer without the compensating effect that would otherwise be produced by the freezing mixture, with the result that the observed zero point may be several hundredths of a degree too high. The error thus introduced is not, as might be supposed, compensated for by determining the freezing point of the solution under exactly the same conditions, since solutions exhibit no tendency to form ice films, thus differing in this respect from distilled water.

The formation of ice films in distilled water can be completely prevented by regulation of the bath temperature so that freezing point and convergence temperature coincide, combined with efficient stirring.

The error due to super-cooling may be very large, and would appear to have been overlooked by the majority of investigators who have dealt with the freezing point of milk, with the exception of Bordas and Génin. The true freezing point of a dilute aqueous solution is that temperature at which it is in exact equilibrium with ice. If the solution is cooled below its freezing point, and a minute fragment of ice then introduced, formation of ice occurs in the liquid, and the temperature immediately rises to a point which approximates to the true freezing point. Obviously the separation of a certain quantity of ice implies a corresponding concentration of the solution, and the observed freezing point will not be that of the original solution, but of a more concentrated

one. It has been shown by Raoult that the effect of super-cooling on the freezing point may be expressed by the following equation:—

$$C^1 = C + K C S \text{ or } K = \frac{C^1 - C}{C S}$$

Where C^1 = the observed depression of the freezing point.

C = the true depression (Δ).

S = degrees of super-cooling.

K = a constant determined experimentally.

The value of K for any particular dissolved substance is found by determining the freezing point for different degrees of super-cooling. If the values found are plotted as ordinates, and the degrees of super-cooling as abscissae, an approximately straight line is obtained from which the true freezing point for super-cooling *nil* may be found. K is then calculated from the above formula. The value of K varies slightly for different solutions. According to Raoult it is approximately 0.013 to 0.014 for potassium chloride, and 0.015 for cane sugar. From the Table on page 11 it will be seen that the mean of the values obtained for cane sugar is 0.015, and similar experiments on milk gave a value of 0.017.

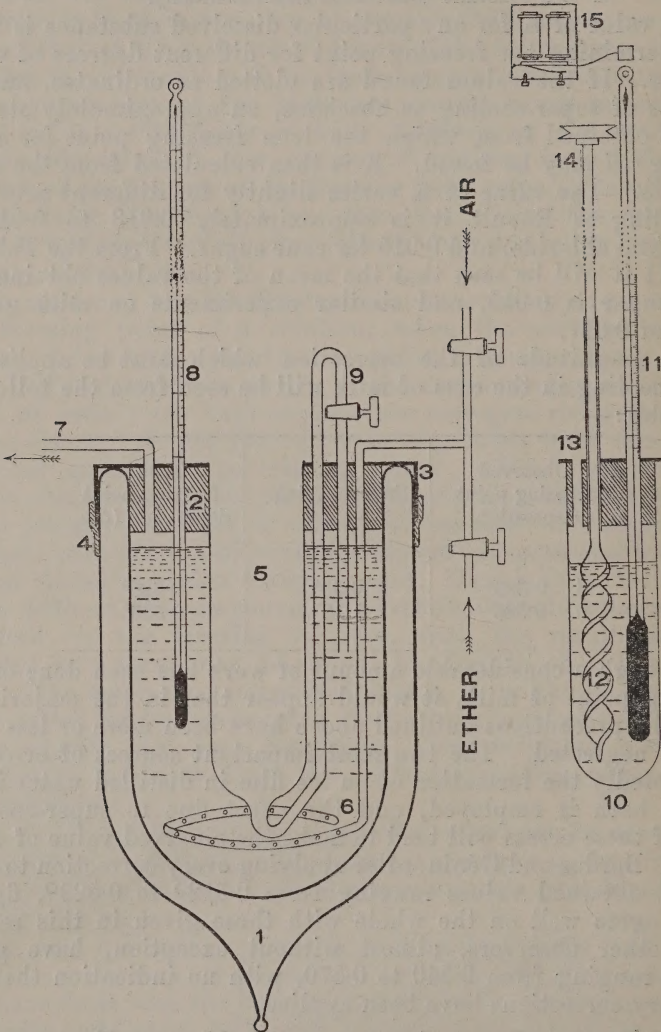
The magnitude of the correction which must be applied for super-cooling in the case of milk will be seen from the following examples:—

Observed freezing point depression.	Super-cooling.	True freezing point depression (Δ).
i. 0.530°	0.55°	0.525°
ii. 0.535°	1.10°	0.525°

Although a considerable amount of work has been done on the freezing point of milk, it would appear that in the majority of cases the precautions outlined above have been more or less completely neglected. The two most important sources of error are undoubtedly the formation of an ice film in distilled water if too cold a bath is employed, and the effect due to super-cooling. Both of these errors will tend to make the observed value of Δ too great. Bordas and Génin, after applying every correction to their results, obtained values ranging from 0.512° to 0.529°, figures which agree well on the whole with those given in this report, while other observers, almost without exception, have given figures ranging from 0.540 to 0.570, with no indication that the necessary corrections have been applied.

III. DESCRIPTION OF APPARATUS AND METHOD EMPLOYED.

The apparatus employed in these experiments was in principle the same as that used by Raoult in his more recent work, the necessary low temperature being attained by the passage of a current of dry air through methylated ether. Certain modifications of the original apparatus were made with a view to effecting an economy of ether and shortening the time required for a determination.



A Dewar vacuum vessel (1) of 10 cm. internal diameter, and 16 cm. internal depth, was closed with a large cork disk (2) about one inch thick, glued to a metal plate. The outer edge of the plate was soldered to a metal ring (3), and the cover thus formed could be made airtight by means of the rubber ring (4). Through the middle of the cork disk passed a glass tube (5) of 3.8 cm. internal diameter, an airtight junction with the metal plate being effected by ordinary glue cement. The cork disk also carried a copper inlet tube for ether and air (6), the lower portion of which was bent round and perforated with a number of small holes, an outlet tube (7), a thermometer (8), graduated into 0.01° , and a siphon (9) connected with the lower end of the tube (5), and so designed that ether could be allowed to run into (5) from the Dewar vessel or drawn out as desired.

The freezing tube (10) was a wide test tube, 3.5 cm. internal diameter, of thin glass. It was closed by a cork, one inch in thickness, carrying the thermometer (11), a spiral glass stirrer (12), and a small opening (13) provided with a stopper. It also carried a small additional thermometer, not shown in the figure, graduated into 0.1° , and serving to indicate the degree of supercooling of the solution.

The freezing point determinations are carried out with this apparatus in the following manner:—

The outlet tube (7) is connected with a water pump, and methylated ether, previously dried with granulated calcium chloride, drawn into the Dewar vessel until the latter is full. About 60 c.c. of the liquid, of which the freezing point is to be determined, are introduced into the freezing tube (10), which is closed with the cork and placed in the tube (5). Ether is allowed to flow from the Dewar vessel through the siphon (9) into the space between the tubes, and the stirrer is connected with the rotating spindle (14), driven by a small electric motor. The supply of ether is shut off and a rapid current of dry air drawn through the Dewar vessel. As the ether evaporates it is replenished by shutting off the air supply and opening the tap connecting with the ether supply. In this way the temperature of the ether bath can be lowered to -3° or -4° in a very short time (15 to 20 minutes). The ether in the space between the tubes (5) and (10) serves as a heat conducting medium, and the temperature of the solution in (10) is thereby lowered far more rapidly than would be the case were the space between the two tubes filled with air. The stirrer is rotated at a constant speed. Preliminary experiments with water containing crystals such as benzoic acid or naphthalene in suspension, will indicate the speed at which the most effective stirring is attained. With the particular form of stirrer used in these experiments the most efficient agitation was reached at a speed of 1,300 revolutions per minute. With a stirrer of larger surface such as was used by Raoult, a much lower speed would be sufficient. It is probable that a high speed of rotation, and consequently a high mechanical production of heat, is of advantage for two reasons—firstly, slight variations in the speed of the motor will have relatively less effect upon the total heat produced by stirring, and secondly, the difference

between the convergence temperature and the bath temperature will be increased, thus reducing the error caused by slight variation in the bath temperature.

When the solution in (10) has reached the desired degree of super-cooling, *i.e.* about 0.5° below the expected freezing point, the air supply is shut off and the tap on the siphon tube (9) opened. The ether is thus drawn out from the space between the tubes (5) and (10), being replaced by air. The tap on the siphon tube is closed, and ether at room temperature drawn into the Dewar vessel until the temperature of the bath has risen to a point approximately 0.24° below the expected freezing point. The degree of super-cooling is observed, and a minute fragment of ice immediately introduced through the opening (13). The small electric hammer (15) is set in motion, and the point to which the mercury of the thermometer (11) rises observed through a telescope at a distance of a few feet, the temperature of the bath being kept approximately constant by drawing air or ether through it as required. Care must be taken that the thermometer (11) is vertical, and that the telescope through which the observation is taken is level, otherwise considerable errors due to parallax may occur. With the particular thermometer used the correct reading was reached in less than a minute, and the observed freezing point remained constant for an indefinite time, provided that the bath temperature and the speed of rotation did not alter.

The freezing points of cane sugar solutions of known strengths.

The following table gives the results obtained with cane sugar solutions of known concentration compared with values calculated from a formula given by Raoult as a result of his own determinations. For small depressions of the freezing point, the differences due to variable degrees of super-cooling are not sufficiently great to enable the value of K to be deduced with any degree of certainty, so that, except in five cases in which K was determined experimentally, Raoult's value of 0.015 has been taken.

Weight in grm. of cane sugar in 100 grm. water.	Super- cooling.	Observed depression of freezing point.	Experimental values of constant K.	Freezing point corrected for super- cooling and emergent column.	Calculated freezing point $C=342-0.99 P$ (Raoult).	Difference between experimental and calculated values.
1.1526	0.43°	0.065°	[0.015]	-0.0645°	-0.0635°	+0.001°
2.0080	0.95°	0.1145°	[0.015]	-0.113°	-0.1105°	+0.0025°
3.0160	0.70°	0.1685°	[0.015]	-0.1665°	-0.1665°	± 0.000°
4.0141	0.95°	0.2245°	[0.015]	-0.2205°	-0.222°	-0.0015°
5.0583	0.44°	0.285°	[0.015]	-0.282°	-0.281°	+0.001°
6.0795	0.40°	0.3435°	[0.015]	-0.340°	-0.339°	+0.001°
7.0211	1.67°	0.401°	0.014	-0.390°	-0.392°	-0.002°
	0.87°	0.3965°		-0.390°		
8.0247	1.02°	0.456°	0.013	-0.448°	-0.450°	-0.002°
9.0417	0.52°	0.453°		-0.448°		
	0.95°	0.518°	[0.015]	-0.509°	-0.5035°	+0.0005°
10.0581	1.15°	0.577°	0.014	-0.5655°	-0.567°	-0.001°
	0.38°	0.571°		-0.5665°		
11.0732	1.38°	0.6385°	0.016	-0.6235°	-0.626°	-0.0025°
	0.44°	0.6295°		-0.6235°		
	1.69°	0.738°	0.017	-0.718°	-0.7175°	+0.001°
12.6276	0.50°	0.723°		-0.719°		

The differences between the corrected freezing points and the values calculated from Raoult's formula are in no case greater than $\pm 0.0025^\circ$. The differences may be due partly to slight inaccuracies in the scale of the thermometer, and partly to errors in the determination of the zero point. The ice which separates from distilled water exhibits a tendency to ball together into a more or less solid lump, whereas with solutions the ice crystals remain evenly distributed through the liquid. Owing to the reduction of the effective surface between ice and water which is thus brought about, equilibrium is not so readily established, and it is sometimes difficult to obtain absolutely constant readings for the zero point.

Determination of the super-cooling correction (K) for milk.

The freezing point of a sample of milk was determined as accurately as possible for four different degrees of super-cooling, a fresh portion of the milk being taken for each determination.

The following results were obtained:—

<i>Super-cooling.</i>					<i>Observed depression of freezing point.</i>
1.73°	...	...	...	...	0.540°
1.24°	...	...	...	...	0.535°
0.54°	...	...	...	...	0.530°
0.20°	...	...	...	...	0.526°

If the observed values for the freezing point are plotted as ordinates, and the degrees of super-cooling as abscissae, an approximately straight line is obtained, from which the true freezing point depression for super-cooling *nil* is found to be 0.5245° . The value of K is then found from the formula $K = \frac{C - C}{C S}$ (page 7) to be 0.017.

It has been stated (Richmond, Dairy Chemistry, 1899, page 155), that when milk is partially frozen the solid portion does not consist of pure ice, but contains a certain proportion of the original milk solids. This is no doubt the case when milk in bulk is partially frozen, *e.g.* in a churn, but when a small degree of super-cooling is produced in milk which is rapidly stirred, it would appear that the solid substance separating out is either pure ice or ice containing fat and proteins only. If the ice contained lactose or soluble salts, the super-cooling correction K would be smaller than that found for solutions of potassium chloride and cane sugar, whereas in reality it is slightly greater.

IV. RESULTS OBTAINED WITH MILK SAMPLES FROM DIFFERENT SOURCES.

In the appended tables are given the results of complete analyses and freezing point determinations of 141 milk samples. Of these samples 111 are from single cows (shorthorns), and 30 are samples of the mixed evening milk of herds. I am indebted to Mr. Cecil Revis, chief chemist to Messrs. Welford and Sons, Ltd., for the greater number of the samples, and to Mr. J. Golding, F.I.C., of University College, Reading, for a series of 33 samples from the Reading Agricultural College Experimental farm.

The utmost care has been taken to ensure the genuineness of every sample received. The samples from single cows were sent direct from the farm in bottles, and the milk was neither strained nor pasteurised. Samples 1 to 54 were mainly from cows in the later stages of the lactation period, which were being dried off. The same remark applies to some of the samples from Reading, a few of which, *e.g.*, Nos. 105 and 111, were extraordinarily abnormal as regards chemical composition. One of them (No. 111) bore very little resemblance to normal milk, being in a clotted and stringy condition. The cows from which these samples were obtained were drying off, but were apparently in perfect health. In view of the interest attaching to the values obtained for the freezing point, all these abnormal samples have been included in the series.

The values given for the freezing points have been subjected to all the necessary corrections indicated on pages 3 to 7, and may be taken as representing the true freezing point depressions with a probable accuracy of about $\pm 0.002^{\circ}$. In the rest of the analytical work the following methods were used:—

- i. *Fat*. Mean of two determinations by the Leffmann-Beam centrifugal method, the bottles being previously standardised by comparison with the results obtained by Gottlieb's method.
- ii. *Total solids*. 5 grms. of the sample evaporated in flat-bottomed nickel dishes of 7 c.m. diameter and heated in a steam oven to constant weight.
- iii. *Ash*. 10 grms. of the sample evaporated in porcelain and ashed in an electrically heated muffle at as low a temperature as possible. Mean of two determinations.
- iv. *Specific gravity*. Taken at 15.5° in a 50 c.c. specific gravity bottle.
- v. *Lactose*. By the polarimeter, using Richmond and Boseley's dilution formula.
- iv. *Proteins*. Calculated from the aldehyde figure (Richmond). Mean of two determinations.
- vii. *Acidity*. Mean of two determinations by Richmond and Huish's titration method using the Rosaniline standard (R.S.).

In the case of many of the abnormal samples met with from single cows, the results were confirmed by other methods.

Samples of Milk from Single Cows (Welford's Dairy).

Sample No.	Date.	Cow No.	Time of milking.	Quantity of milk in pounds.	Acidity (R. S.).	Specific gravity.	Total Solids, per cent.	Fat, per cent.	Non-fat solids, per cent.	Lactose, per cent.	Proteins (aldehyde figure $\times 0.170$), per cent.	Ash, per cent.	Depression of Freezing point (Δ) (corrected).
1	6/V/14	5	5.0 A.M.	4 $\frac{1}{2}$	17.9°	1.0317	14.50	5.4	9.10	4.01	3.99	0.82	0.528°
2	"	6	5.0 "	12	17.7°	1.0306	13.84	5.1	8.74	4.44	3.31	0.68	0.528°
3	"	7	5.0 "	10 $\frac{1}{2}$	20.4°	1.03215	11.84	3.0	8.84	4.15	3.61	0.76	0.526°
4	"	4	5.0 "	12	19.3°	1.0321	12.18	3.4	8.78	4.24	3.57	0.75	0.536°
5	9/V/14	1	5.0 A.M.	4	17.1°	1.0302	11.53	3.2	8.33	3.79	3.50	0.80	0.540°
6	"	2	5.0 "	11	26.6°	1.0353	13.73	4.0	9.73	4.75	4.05	0.82	0.553°
7	"	3	5.0 "	18	28.3°	1.0352	12.55	3.0	9.55	4.58	3.91	0.80	0.544°
8	"	3	5.0 "	9	20.0°	1.0350	12.27	2.7	9.57	4.50	3.91	0.85	0.545°
9	11/V/14	6	3.30 P.M.	—	17.0°	1.0320	15.69	6.25	9.44	4.30	4.06	0.82	0.538°
10	"	9	3.15 "	—	16.5°	1.0312	15.04	6.1	8.94	4.64	3.50	0.70	0.547°
11	"	7	3.15 "	—	20.6°	1.0329	12.78	3.6	9.18	4.38	3.84	0.75	0.539°
12	"	8	3.30 "	—	16.6°	1.0324	12.49	3.6	8.89	4.25	3.65	0.77	0.550°
13	13/V/14	1	4 P.M.	—	17.2°	1.0298	11.97	3.8	8.17	3.67	3.57	0.76	0.530°
14	"	2	3.30 "	—	27.5°	1.0326	13.74	4.4	9.34	4.62	3.83	0.77	0.528°
15	"	3	3.45 "	—	26.2°	1.0333	13.47	4.3	9.14	4.79	3.75	0.72	0.526°
16	"	4	3.45 "	—	19.0°	1.0325	13.18	3.95	9.23	4.40	3.80	0.78	0.522°
17	16/V/14	5	5.45 A.M.	—	15.8°	1.0326	14.79	5.5	9.29	4.18	4.13	0.87	0.550°
18	"	6	5.15 "	—	16.7°	1.0331	12.11	3.15	8.96	4.72	3.50	0.70	0.5475°
19	"	7	5 "	—	19.4°	1.0336	12.67	3.4	9.27	4.54	3.79	0.78	0.557°
20	"	8	5.30 "	—	16.2°	1.0326	12.41	3.55	8.86	4.27	3.59	0.77	0.547°
21	19/V/14	1	5.45 A.M.	—	17.1°	1.0300	10.96	2.9	8.06	3.66	3.48	0.79	0.534°
22	"	1	5 "	—	28.6°	1.0341	13.07	3.65	9.42	4.65	4.03	0.78	0.532°
23	"	2	5.30 "	—	26.0°	1.0350	12.60	3.05	9.55	4.89	3.86	0.76	0.538°
24	"	3	5.15 "	—	18.6°	1.0340	11.64	2.5	9.14	4.24	3.86	0.84	0.532°
25	20/V/14	4	3.45 P.M.	3 $\frac{1}{2}$	17.0°	1.0316	15.48	6.25	9.23	4.25	4.06	0.81	0.530°
26	"	5	3.15 "	9	18.1°	1.0307	14.38	5.5	8.88	4.70	3.35	0.69	0.535°
27	"	6	3 "	8	21.4°	1.0326	12.51	3.55	8.96	4.52	3.62	0.74	0.532°
28	"	7	3.30 "	8	18.4°	1.0328	12.62	3.7	8.92	4.42	3.55	0.76	0.538°
29	22/V/14	8	3.15 P.M.	3	17.6°	1.0303	11.50	3.2	8.30	4.02	3.40	0.77	0.530°
30	"	1	3 "	11	28.0°	1.0334	14.00	4.5	9.50	4.71	3.91	0.76	0.528°
31	"	2	3.30 "	17 $\frac{1}{2}$	26.5°	1.0331	14.16	4.75	9.41	4.84	3.74	0.72	0.532°
32	"	3	3.45 "	8 $\frac{1}{2}$	20.7°	1.0338	12.96	3.7	9.26	4.62	3.79	0.81	0.527°
33	26/V/14	4	5.45 A.M.	4	16.6°	1.0294	10.38	2.9	8.08	3.75	3.42	0.77	0.531°
34	"	1	5.15 "	20	26.9°	1.0346	12.31	2.9	9.41	4.86	3.77	0.77	0.5365°
35	"	3	5.30 "	11 $\frac{1}{2}$	20.7°	1.0324	12.08	3.2	8.88	4.44	3.57	0.76	0.534°
36	"	7	5.30 "	11	18.2°	1.0330	12.21	3.2	9.01	4.58	3.57	0.75	0.5385°
37	28/V/14	8	5 A.M.	15	28.5°	1.0341	12.03	3.65	9.38	4.73	3.89	0.79	0.533°
38	"	2	5.15 "	11	20.5°	1.0341	12.67	3.3	9.37	4.60	3.86	0.81	0.528°
39	"	5	5.45 "	5	17.6°	1.0326	13.55	4.4	9.15	4.47	3.77	0.80	0.537°
40	"	6	5.30 "	10	16.4°	1.0316	13.19	4.35	8.84	4.75	3.26	0.67	0.537°

Sample No.	Date.	Cow No.	Time of milking.	Quantity of milk in pounds.	Acidity (H. S.).	Specific gravity.	Total solids, per cent.	Fat, per cent.	Non-fat solids, per cent.	Lactose, per cent.	Proteins (aldehyde figure $\times 0.170$), per cent.	Ash, per cent.	Depression of Freezing point (Δ) (corrected).
41	29/1/14	1	3 P.M.	13	15.9°	1.0296	11.63	3.5	8.13	3.73	3.41	0.77	0.5335°
42	"	2	3.30	15	24.2°	1.0338	13.47	4.05	9.42	4.91	3.59	0.72	0.533°
43	"	7	3.45	7	20.8°	1.0317	12.30	3.55	8.75	4.30	3.37	0.73	0.533°
44	"	8	3.15	7	19.4°	1.0334	12.67	3.6	9.07	4.50	3.52	0.74	0.537°
45	1/2/14	2	3 P.M.	10	20.3°	1.0349	13.84	4.2	9.64	4.66	4.05	0.84	0.550°
46	"	4	3.15	8	20.1°	1.03415	13.38	3.95	9.43	4.62	3.71	0.81	0.539°
47	"	2	3.30	7	17.5°	1.0336	14.08	4.65	9.43	4.45	3.91	0.84	0.543°
48	"	9	3.45	7	16.7°	1.0311	14.73	5.75	8.98	4.66	3.33	0.70	0.552°
49	4/2/14	3	5 A.M.	19	25.6°	1.0352	12.91	3.4	9.51	5.06	3.83	0.76	0.544°
50	"	7	5.15	11	20.5°	1.0325	11.46	3.7	8.76	4.39	3.50	0.76	0.5365°
51	"	8	5.30	9	17.5°	1.0331	12.19	3.2	9.00	4.32	3.67	0.78	0.536°
52	6/2/14	2	5 A.M.	15	28.7°	1.0353	13.11	3.4	9.71	4.79	4.13	0.80	0.5435°
53	"	4	5.30	11	20.3°	1.0347	12.81	3.3	9.51	4.59	3.96	0.81	0.539°
54	"	7	5.15	11	20.1°	1.0321	12.00	3.2	8.80	4.40	3.47	0.76	0.541°
55	27/4/14	9	4.45 A.M.	18	22.5°	1.0331	13.08	4.0	9.08	5.00	3.33	0.69	0.5325°
56	"	10	4.45	19	27.2°	1.0331	13.06	3.85	9.21	5.00	3.25	0.77	0.535°
57	"	11	5.0	20	22.0°	1.0312	11.68	3.15	8.53	4.48	3.18	0.78	0.532°
58	"	12	5.15	20	27.3°	1.0329	13.58	4.4	9.18	4.97	3.47	0.76	0.5335°
59	29/4/14	9	3 P.M.	16	21.2°	1.0324	13.47	4.3	9.17	5.08	3.33	0.66	0.5265°
60	"	10	3.15	15	27.4°	1.0314	13.40	4.5	8.90	4.83	3.26	0.73	0.521°
61	"	11	3	14	20.4°	1.0306	11.33	3.05	8.28	4.54	2.96	0.71	0.526°
62	"	12	3.15	16	25.5°	1.0308	14.48	5.6	8.88	4.96	3.01	0.76	0.524°
63	4/5/14	13	4.45 A.M.	17	20.3°	1.0269	16.26	7.65	8.61	4.48	2.91	0.67	0.5265°
64	"	14	5	20	24.1°	1.03405	12.26	3.05	9.21	5.05	3.23	0.74	0.546°
65	"	15	5.15	16	20.2°	1.0319	11.30	2.6	8.70	4.94	2.92	0.70	0.5285°
66	"	16	5.30	16	18.8°	1.0326	12.31	3.35	8.96	4.77	3.42	0.74	0.537°
67	6/5/14	13	3 P.M.	18	21.9°	1.0295	12.81	4.4	8.41	4.87	2.63	0.66	0.520°
68	"	14	3.15	13	24.8°	1.0317	12.85	4.0	8.85	4.85	3.16	0.71	0.5335°
69	"	15	3.30	12	20.0°	1.0323	11.43	2.7	8.73	4.82	2.97	0.70	0.533°
70	"	16	3.45	18	17.5°	1.0281	15.42	6.7	8.72	4.50	3.11	0.69	0.5195°
71	11/5/14	17	5 A.M.	13	18.7°	1.0331	12.76	3.55	9.21	4.60	3.60	0.78	0.5475°
72	"	18	5.15	17	23.1°	1.0333	12.84	3.7	9.14	4.80	3.45	0.82	0.546°
73	"	19	5.30	14	21.5°	1.0329	12.25	3.3	8.95	5.22	2.92	0.68	0.543°
74	"	20	4.45	15	19.6°	1.0333	12.71	3.5	9.21	5.16	3.06	0.68	0.5465°
75	13/5/14	17	3.15 P.M.	11	17.9°	1.0317	13.62	4.5	9.12	4.48	3.06	0.73	0.530°
76	"	18	3	14	21.0°	1.0330	12.56	3.5	9.06	4.77	3.47	0.80	0.521°
77	"	19	3.15	11	22.0°	1.03205	12.79	3.85	8.94	5.00	3.19	0.70	0.524°
78	"	20	3	13	19.3°	1.0318	12.90	4.0	8.90	4.92	2.87	0.66	0.525°

Samples of Milk from Single Cows (Reading Agricultural College).

Sample No.	Date.	Cow No.	Time of milking.	Quantity of milk in pounds.	Acidity (R. S.).	Specific gravity.	Total Solids, per cent.	Fat, per cent.	Non-fat solids, per cent.	Lactose, per cent.	Proteins (aldehyde figure $\times 0.170$), per cent.	Ash, per cent.	Depression of Freezing point (Δ) (corrected).
79	12/2/14	21	4.15 P.M.	10	16.4°	1.0322	12.73	3.95	8.78	4.63	3.31	0.74	0.5365°
80	"	22	3.15 "	16 $\frac{1}{2}$	23.1°	1.0328	13.07	4.1	8.97	4.98	3.21	0.72	0.5385°
81	"	23	3.45 "	7	23.4°	1.0347	15.63	5.7	9.93	5.08	3.84	0.73	0.539°
82	"	24	3.30 "	18 $\frac{1}{2}$	19.6°	1.0324	13.10	4.2	8.90	4.90	3.14	0.70	0.530°
83	"	25	4 "	8	18.2°	1.0321	13.11	4.4	8.71	4.72	3.30	0.69	0.524°
84	"	26	4 "	14	27.0°	1.0323	13.36	4.5	8.86	4.80	3.35	0.74	0.531°
85	"	27	3.45 "	3	26.0°	1.0355	15.63	5.5	10.13	4.47	4.45	0.89	0.552°
86	"	28	4 "	10	18.3°	1.0325	12.76	3.9	8.86	4.68	3.38	0.72	0.525°
87	17/2/14	21	7 A.M.	15	17.3°	1.0334	11.69	2.85	8.84	4.70	3.38	0.76	0.546°
88	"	25	6.30 "	12 $\frac{1}{2}$	20.0°	1.0337	12.43	3.25	9.18	4.91	3.33	0.71	0.541°
89	"	29	7.15 "	3 $\frac{1}{2}$	17.1°	1.0301	19.08	3.8	9.68	3.81	4.16	0.83	0.5505°
90	"	30	7.15 "	9	18.4°	1.0358	13.77	9.4	9.97	4.68	4.22	0.81	0.538°
91	"	31	6.30 "	19	21.0°	1.0338	12.44	3.3	9.14	5.17	3.14	0.72	0.553°
92	"	32	6.45 "	15 $\frac{1}{2}$	20.8°	1.0337	12.62	3.45	9.17	4.95	3.45	0.78	0.545°
93	18/2/14	21	3.45 P.M.	9 $\frac{1}{2}$	16.9°	1.0306	12.37	3.8	8.57	4.59	3.15	0.71	0.524°
94	"	22	3.30 "	15 $\frac{1}{2}$	22.7°	1.0325	12.92	4.0	8.92	4.83	3.06	0.71	0.531°
95	"	25	4 "	8 $\frac{1}{2}$	19.0°	1.0325	13.19	4.15	9.04	4.80	3.31	0.70	0.5285°
96	"	29	3.30 "	—	17.4°	1.0296	16.41	7.2	9.21	3.94	3.65	0.81	0.544°
97	"	30	4 "	4	17.3°	1.0344	14.95	5.0	9.95	4.68	4.49	0.79	0.539°
98	"	31	3.45 "	13 $\frac{1}{2}$	20.0°	1.0306	13.61	4.9	8.71	4.89	2.94	0.67	0.5275°
99	"	32	4 "	10 $\frac{1}{2}$	20.0°	1.0324	12.87	3.85	9.02	4.82	3.30	0.74	0.524°
100	19/2/14	22	3.30 P.M.	14 $\frac{1}{2}$	21.4°	1.0335	13.40	4.0	9.40	5.05	3.31	0.75	0.546°
101	"	23	3.30 "	4	18.7°	1.0364	12.23	2.35	9.68	4.44	3.94	0.84	0.532°
102	"	24	3.45 "	16	17.7°	1.0332	13.16	4.0	9.16	4.90	3.21	0.74	0.512°
103	"	26	4 "	13 $\frac{1}{2}$	27.1°	1.0350	13.18	3.4	9.78	4.00	3.48	0.77	0.550°
104	"	28	3.45 "	10 $\frac{1}{2}$	17.3°	1.0314	13.89	4.9	8.99	4.50	3.31	0.74	0.5325°
105	25/2/14	23	6.45 A.M.	6	9.0°	1.0201	19.15	11.6	7.55	5.15	4.01	0.99	0.546°
106	"	24	6.30 "	25 $\frac{1}{2}$	18.7°	1.0346	12.65	3.3	9.35	5.15	3.30	0.73	0.545°
107	"	26	6.45 "	23 $\frac{1}{2}$	26.5°	1.0354	12.58	3.15	9.43	5.12	3.37	0.79	0.558°
108	"	27	6.45 "	—	19.7°	1.0356	13.84	3.75	10.09	4.41	4.27	0.94	0.5485°
109	"	29	6.45 "	3 $\frac{1}{2}$	14.9°	1.0331	12.79	3.4	9.39	3.41	4.27	0.91	0.537°
110	"	32	7 "	—	20.6°	1.0334	12.44	3.35	9.09	4.88	3.28	0.79	0.5365°
111	11/3/14	27	4 P.M.	—	20.7°	—	11.13	1.29	9.84	0.71	6.26	1.29	0.552°

*Samples of Mixed Evening Milk from different farms, mainly in Wilts, Berks, Somerset, Dorset and Oxon.
(Welford's Dairy).*

Sample No.	Date.	Acidity (R. S.).	Specific gravity.	Total solids, per cent.	Fat, per cent.	Non-fatty solids, per cent.	Lactose, per cent.	Proteins (aldehyde figure $\times 0.170$), per cent.	Ash, per cent.	Depression of Freezing point (Δ) (corrected).
112	23/8/14	17.7°	1.0331	12.63	3.5	9.13	4.65	3.45	0.77	0.53°
113	"	18.3°	1.0318	12.29	3.6	8.69	4.70	3.14	0.72	0.514°
114	"	21.6°	1.0327	14.05	4.75	9.30	4.85	3.48	0.76	0.5345°
115	"	20.2°	1.0315	11.93	3.3	8.63	4.55	3.25	0.76	0.521°
116	"	21.1°	1.0337	12.18	3.1	9.08	4.75	3.56	0.75	0.523°
117	"	19.4°	1.0332	12.85	3.75	9.10	4.79	3.52	0.75	0.5245°
118	"	20.6°	1.0323	12.71	3.7	9.01	4.61	3.43	0.75	0.529°
119	"	20.5°	1.0323	12.70	3.7	9.00	4.63	3.33	0.75	0.531°
120	"	21.0°	1.0319	13.02	4.15	8.87	4.60	3.35	0.75	0.5275°
121	"	19.8°	1.0318	12.30	3.5	8.80	4.77	3.13	0.70	0.5185°
122	"	20.9°	1.0326	12.78	3.8	8.98	4.63	3.55	0.75	0.528°
123	"	18.9°	1.0310	12.05	3.4	8.65	4.60	3.09	0.71	0.525°
124	30/8/14	20.2°	1.0326	11.80	2.9	8.90	4.88	3.20	0.74	0.530°
125	"	24.7°	1.0345	12.57	3.2	9.37	4.99	3.57	0.78	0.5375°
126	"	18.6°	1.0320	12.23	3.4	8.83	4.80	3.16	0.72	0.5195°
127	"	20.6°	1.0341	13.88	4.25	9.63	4.78	3.88	0.76	0.5375°
128	"	19.9°	1.0318	13.04	4.1	8.94	4.57	3.42	0.75	0.534°
129	"	18.7°	1.0323	12.16	3.3	8.86	4.70	3.14	0.76	0.5325°
130	"	18.9°	1.0302	12.48	4.0	8.48	4.50	3.18	0.72	0.522°
131	2/4/14	18.0°	1.0312	11.83	3.4	8.43	4.50	3.01	0.71	0.528°
132	"	20.8°	1.0319	13.12	4.1	9.02	4.65	3.42	0.72	0.530°
133	"	18.4°	1.0305	12.69	4.1	8.59	4.55	3.11	0.68	0.517°
134	"	17.5°	1.0300	11.94	3.6	8.34	4.29	3.16	0.68	0.517°
135	"	19.4°	1.0309	12.95	4.3	8.65	4.87	3.01	0.70	0.517°
136	6/4/14	19.9°	1.0333	12.35	3.25	9.10	5.00	3.30	0.72	0.5305°
137	"	19.5°	1.0320	13.05	4.1	8.95	4.81	3.37	0.72	0.5235°
138	"	20.6°	1.03285	12.93	3.7	9.23	4.91	3.42	0.77	0.528°
139	"	10.8°	1.0316	11.88	3.15	8.73	3.81	4.25	0.73	0.5305°
140	"	20.1°	1.0318	13.07	4.1	8.97	4.99	3.16	0.72	0.5285°
141	"	17.7°	1.0313	12.17	3.5	8.67	4.72	3.08	0.72	0.5255°

V. DISCUSSION OF RESULTS.

From the foregoing results it will be seen that the values of Δ given by samples from single cows, vary from 0.5195° to 0.558° , with a mean of 0.5366° , and of mixed milk samples from 0.514° to 0.5375° with a mean of 0.5266° . The samples of mixed milk were all from evening milkings, and were obtained in March and April, while the samples from single cows were evenly distributed over morning and evening milkings, and were obtained partly in January and February and partly in April and May. The average value obtained for the morning milk of single cows is 0.540° and for the evening milk 0.533° . In consequence of the relatively low values given by the series of mixed evening milks, some experiments were carried out with a view to ascertaining whether the freezing point of a mixed milk is necessarily the same as the mean of the values given by the individual samples before mixing. It is conceivable that molecular rearrangements or physical changes might take place on mixing two or more samples of different character which might have some influence upon the freezing point. No evidence could be obtained, however, that such changes occur, the freezing point of the mixture being invariably the same, within the limits of experimental error, as that calculated from the values found for the separate constituents.

It is evident that the evening milk shows, on the whole, a lower value for Δ than the morning milk, and also that in the months of March, April and May the values obtained are, in general, lower than during January and February. Until data extending over a whole year are available, it is impossible to say whether these results represent the extreme limits of seasonal variation. It is also possible that under different farming conditions the difference between the morning and evening milk may be increased. Thus certain preliminary experiments carried out towards the end of November, 1913, upon six samples of milk from a private herd of Jersey cows and one sample of mixed evening milk, gave the following results:—

					Morning milk.	Evening milk.
Jersey cow	No. 1	...	...	...	0.539°	0.521°
"	No. 2	...	...	...	0.547°	0.509°
"	No. 3	...	...	...	0.541°	0.523°
Mixed milk (breed not ascertained; probably shorthorn)	...	...	...	...	—	0.508°

Two out of these seven samples, therefore, gave values slightly lower than the lowest recorded during January to May. As far as could be ascertained the samples were undoubtedly genuine, and no special reason could be found for these two instances of a somewhat abnormally small depression of the freezing point. It would appear, therefore, that the value of 0.514° is only tentatively to be regarded as the lower limit of the freezing point until further results are available covering all seasons of the year and different conditions of feeding, intervals between hours of milking, etc.

The following table gives the highest, lowest, and average values for the various constituents observed in the 141 analyses recorded above.

In the case of samples 105 and 111, the values for lactose and ash, which are obviously extremely abnormal, have been omitted, also the value for protein given by sample 111. At the same time all the values obtained for the freezing point, with the exception of a few obtained for colostrum, have been included, and it is noteworthy that, however abnormal the sample from a chemical point of view, the freezing point seems to be almost unaffected. As a matter of fact the freezing point of colostrum would appear to be practically the same as that of normal milk, four samples that were examined giving the values 0.5275° , 0.547° , 0.549° and 0.5435° . From the figures given under the heading "per cent. variation from average," the relative extent to which the different constituents are found to vary from the average is readily seen. Thus the figures $+12.2\%$ -10.7% represent the extent to which the highest and lowest values differ from the average of all the analyses, when this average is made equal to 100.

	Non-fatty solids.	Lactose.	Proteins.	Ash.	Freezing point depression. (Δ).
Highest	10.13	5.22	4.49	0.94	0.558°
Average	9.03	4.61	3.43	0.75	0.5345°
Lowest	8.06	3.41	2.63	0.66	0.514°
Per cent. variation {	$+12.2\%$	$+13.2\%$	$+30.9\%$	$+25.3\%$	$+4.4\%*$
from average. }	-10.7%	-26.0%	-23.3%	-12.0%	-3.9%

It is evident therefore that, in the 141 samples examined, the relative extent of the variation in the freezing point is considerably less than that of any of the principle constituents, and that the freezing point is therefore the most constant property exhibited by milk. Accordingly, in cases of adulteration, a more correct approximation to the actual amount of added water present should be obtained from a consideration of the freezing point than is possible if the percentage of any *one* constituent, such as non-fatty solids, is considered. It is, however, universally admitted that a correct opinion as to the actual quantity of added water cannot be formed upon an estimation of non-fatty solids alone, and that for this purpose determinations of other constituents in addition, such as total nitrogen and ash, are necessary. Given any particular sample of milk in which the content of non-fatty solids lies between 8.0 and 8.5 per cent., it is a question as to whether a more correct conclusion can be arrived at by the somewhat difficult and tedious determination of the freezing point than by the estimation of lactose, aldehyde figure (or total nitrogen) and ash. In this connection it is interesting to examine in detail some of the actual analytical results obtained:—

* It is noteworthy that if the lower limit of the freezing point depression is taken as being 0.508° (page 18) the percentage variation from the average is only altered from $+4.4$ per cent. to $+4.4$ per cent.
 -3.9 per cent. to -4.9 per cent.

Sample No.	...	...	5	13	21	29	33	41	105	130	131	134	139	61	67
Freezing point depression(Δ)	...	...	0.540°	0.530°	0.534°	0.530°	0.531°	0.5335°	0.546°	0.522°	0.528°	0.517°	0.5305°	0.526°	0.520°
Fat, per cent.	...	...	3.2	3.8	2.9	3.2	2.9	3.5	11.6	4.0	3.4	3.6	3.15	3.05	4.4
Non-fatty solids, per cent.	...	...	8.33	8.17	8.06	8.30	8.08	8.13	7.55	8.48	8.43	8.34	8.73	8.28	8.41
Lactose, per cent. ...	...	...	3.79	3.67	3.66	4.02	3.75	3.73	1.80	4.50	4.50	4.29	3.81	4.54	4.87
Proteins, per cent.	...	...	3.50	3.57	3.48	3.40	3.42	3.41	4.01	3.18	3.01	3.16	4.25	2.96	2.63
Ash, per cent.	...	...	0.80	0.76	0.79	0.77	0.77	0.77	0.99	0.72	0.71	0.68	0.73	0.71	0.66
Ratio lactose protein	...	...	1.08	1.03	1.05	1.18	1.10	1.09	0.45	1.41	1.49	1.36	0.90	1.53	1.85

Normal ratio $\frac{\text{lactose}}{\text{protein}} = 1.40.$

The first six of these were all obtained from one cow, towards the end of the lactation period, and are characterised throughout by a low content of lactose. In view of the perfectly normal figures for proteins and ash, such samples are not likely to be condemned as containing added water, and the additional evidence afforded by the freezing point is unnecessary. Sample 105 is extremely high in fat and ash and low in lactose, the protein content being high. Although the non-fatty solids are only 7.55 per cent., the results of chemical analysis alone are quite sufficient to indicate that the sample is probably genuine, but of extremely abnormal character.

Samples 61 and 67 show abnormally low figures for protein. They were obtained from single cows at the beginning of May, 1914, a period of the year in which deficiency in protein would appear to occur somewhat frequently. Although the ash is low, the comparatively high figures for lactose are in agreement with the fact that the samples are genuine though abnormal. The sample of mixed milk (No. 139), although not below the 8.5 per cent. limit for non-fatty solids, is of interest owing to the abnormal ratio between lactose and protein. The ash and freezing point are quite normal.

In the case of the mixed milks (Nos. 130, 131 and 134), the figures obtained for non-fatty solids, when compared with the legal limit of 8.5 per cent., indicate the presence of at least 0.24, 0.82 and 1.88 per cent. of added water respectively. Proteins and ash are low, and the ratio of lactose to protein does not differ to any great extent from the normal ratio, so that the analytical data would appear to confirm the presence of small amounts of added water. So far as could be ascertained these samples were undoubtedly genuine. If they had been taken under the Sale of Food and Drugs Acts, the question might have arisen whether they were genuine but of abnormal composition, or whether they contained such small amounts of water as would be indicated by comparison with the 8.5 per cent. limit, or whether they were the result of the addition of comparatively large quantities of water to an originally rich milk.

It is in such cases that a consideration of the freezing point may be of value. In the three samples under consideration the values of Δ are, it is true, lower than the average, and do not exclude the possibility of small quantities of water being present, but it is tolerably certain that the samples could not be the result of heavy adulteration of originally rich milks.

In the analysis of official samples taken under the Sale of Food and Drugs Acts, a considerable number of these cases occur. The non-fatty solids are slightly below the 8.5 per cent. limit, and the relative proportions of lactose, proteins and ash are approximately normal. With such samples a freezing point determination may afford valuable supplementary evidence as to whether added water is present, and will give a more accurate indication of the amount than could be obtained from the results of chemical analysis alone.

Simplification of experimental procedure in freezing point determinations.

The experimental difficulties which are met with in arriving at the correct freezing point militate greatly against the practical value of the method. The use of such comparatively complicated apparatus as that shown on p. 8 is quite out of the question, except for purposes of research. These difficulties could be very much reduced, if not wholly eliminated, by dispensing altogether with the determination of the zero point given by distilled water, and comparing the freezing point of the milk sample with that of a solution of 9.495 grms. of pure cane sugar in 100 grms. of water. This solution freezes at exactly -0.5345° , the average freezing point of normal milk. If the two determinations are carried out in precisely the same manner, they will both be affected to a similar extent by the various errors referred to on pages 3 to 7, and the difference between the two results will indicate fairly accurately the true freezing point of the milk sample in question. This amounts, in fact, to a standardisation in one operation of the particular apparatus used, by means of a standard solution of cane sugar, and permits the use of any form of freezing point apparatus that may be available. The bath may be of ice and salt, or any of the usual freezing mixtures, and the temperature as low as -4° to -5° , the important point being that the temperature be kept approximately the same in both determinations. The same degree of super-cooling must be employed both for the cane sugar solution and the milk, and the rate and manner of stirring must be as far as possible the same in both cases. If this mode of working be adopted it should be possible to carry out freezing point determinations on milk samples without undue expenditure of time and with a degree of accuracy very little inferior to that attained by the use of more complicated apparatus.

Effect of development of acidity in the milk.

A great disadvantage of the method from the point of view of the Public Analyst is the rise of the freezing point depression (Δ) with progressive souring of the milk. While the chemical composition of the sample is only altered to a comparatively slight extent by a moderate degree of souring, the freezing point depression may rise to a value approximating 0.700° . It has been claimed by Stoecklin that the original freezing point of the fresh milk may be calculated by taking into account the degree of acidity developed, the formation of 1 gm. of lactic acid per litre corresponding to a rise of 0.050° in the freezing point depression. The following table gives the results obtained with a considerable number of milk samples which were allowed to stand at room temperature for varying periods. The average rise in the freezing point corresponding to the production of 1 gm. of lactic acid per litre is 0.026° to 0.027° , but the individual results obtained are not sufficiently regular to enable an accurate calculation of the original freezing point of any given sample when fresh to be made.

It is moreover impossible, from a simple estimation of acidity using phenolphthalein as indicator, to determine how much of this is due to development of lactic acid, and how much to the original acidity natural to fresh milk. The results given in the tables of analyses on pages 14 to 17, show that the natural acidity of fresh milk, which is in all probability not due to lactic acid at all, may vary within tolerably wide limits.

<i>Development of acidity.</i>			<i>Corresponding rise in the freezing point depression.</i>
0.9°	...	...	Nil.
1.0°	...	...	0.001°
1.8°	...	...	0.0015°
1.9°	...	...	0.0045°
2.7°	...	...	0.003°
3.0°	...	...	0.001°
4.1°	...	...	0.006°
5.6°	...	...	0.013°
6.5°	...	...	0.010°
9.4°	...	...	0.019°
9.9°	...	...	0.021°
10.4°	...	...	0.026°
12.1°	...	...	0.028°
12.5°	...	...	0.033°
16.3°	...	...	0.033°
16.5°	...	...	0.040°
17.6°	...	...	0.038°
21.4°	...	...	0.0555°
26.1°	...	...	0.088°
26.3°	...	...	0.073°
26.9°	...	...	0.062°
29.4°	...	...	0.073°
40.5°	...	...	0.096°
41.3°	...	...	0.098°
44.6°	...	...	0.102°
46.5°	...	...	0.136°
46.8°	...	...	0.114°
55.4°	...	...	0.1175°
55.5°	...	...	0.148°
56.9°	...	...	0.1365°
60.9°	...	...	0.137°
68.3°	...	...	0.163°

It has been shown, however, by Richmond and Huish that the rate of increase of acidity is very slow during the preliminary stages of souring, and in practice it is found that the taste and smell of the sample are a very good guide as to whether or not the freezing point method is applicable. A sample has to smell and taste distinctly sour before the freezing point (Δ) rises as much as 0.002°.

Effect of removal of the fat.

The freezing points of whole milk and of separated milk are practically the same, as will be seen from the results given below, which were obtained on samples from which the fat was removed in a centrifugal machine.

Sample No.	Freezing point depression (Δ).	
	Whole Milk.	Separated Milk.
124	0.530°	0.5295°
125	0.5375°	0.5375°
126	0.5195°	0.5205°
127	0.5375°	0.537°
128	0.534°	0.5335°
129	0.5325°	0.532°

Since the fat in milk is wholly in a state of mechanical suspension, and not in solution, one would not expect that its removal would affect the osmotic pressure of the milk serum in any way.

Effect of sterilisation by heat.

Sterilisation by heat in closed vessels has a slight effect on both the acidity and the freezing point. This is evident from the following results, in which the effect of heating is seen to have caused a slight fall in both values.

	Sample No.	Original acidity R. S.	Acidity after heating R. S.	Difference.	Original freezing point depression.	Freezing point depression after heating.	Difference.
Heated momentarily to boiling under reflux condensers and allowed to cool.	118	20.6°	19.5°	-1.1°	0.529°	0.522°	-.007°
	119	20.5°	18.9°	-1.6°	0.531°	0.525°	-.006°
	120	21.0°	19.3°	-1.7°	0.5275°	0.518°	-.0095°
	121	19.8°	18.9°	-0.9°	0.5185°	0.5125°	-.006°
	122	20.9°	19.5°	-1.4°	0.528°	0.521°	-.007°
	123	18.9°	17.3°	-1.6°	0.525°	0.5195°	-.0055°
	124	20.2°	19.7°	-0.5°	0.530°	0.526°	-.004°
Heated on boiling water bath for 3 hours under reflux condensers.	127	20.6°	19.7°	-0.9°	0.5375°	0.533°	-.0045°
	67	—	—	—	0.520°	0.5175°	-.0025°
	68	—	—	—	0.5335°	0.531°	-.0025°
	69	—	—	—	0.533°	0.531°	-.002°
	70	—	—	—	0.5195°	0.517°	-.0025°
Pasteurised for 20 minutes at 60°.							

Calculation of the amount of added water present.

The amount of added water present in any sample can be calculated from the value of Δ in precisely the same way as from the percentage of non-fatty solids.

Thus, taking 0.535° as the average freezing point depression for pure milk, we have:—

$$W = 100 - \frac{100 \Delta}{0.535}$$

where Δ = observed freezing point depression,
and W = the probable percentage of added water.

This formula is not strictly accurate, no account being taken of the volume occupied by proteins and fat, or of the slightly increased electrolytic dissociation of the dissolved salts on dilution with water (A. A. Bonnema). The errors due to these two causes are, however, slight and tend to compensate each other. They may for practical purposes be neglected.

Strictly speaking, allowance should be made for dissolved salts in the water itself, as it is extremely unlikely that, in practice, milk would be mixed with distilled water. Water containing dissolved salts with a total osmotic pressure equivalent to that exerted by 10 parts NaCl in 100,000 parts of water would freeze at approximately -0.006° . If water of this character were added to milk, and the amount present calculated as distilled water from the above formula, a result about 1 per cent. too low would be obtained, *i.e.*, instead of 10 per cent. added water actually present, 9 per cent. would be found.

In view of the comparatively wide range in the freezing point of genuine milk, this correction would not be of great importance in practice.

Naturally the addition of preservatives or colouring matters will cause a more or less pronounced rise in the value obtained, but the deliberate falsification of the freezing point by the addition of glycerine, salt, sodium bicarbonate, etc., which has been made much of by some writers, appears hardly likely to occur in practice, as these substances would be readily detected by chemical analysis.

It is obviously impossible, as a result of the comparatively limited number of analyses given in this report, to assign definite limits for the milk of different breeds of cows at all seasons of the year and under different conditions. The results obtained, however, would appear to indicate that the method may be of value in certain cases as a confirmatory test for milk samples of doubtful quality. The difficulties met with in measuring freezing points with the degree of accuracy necessary for this purpose are considerable, and do not appear to have been fully recognised in the published work upon the subject. Until a sufficient number of reliable results have been accumulated, it would be unwise to

* The *minimum* amount of added water present would, of course, be found by substituting the value 0.514 for 0.535 in the formula.

attach too much importance to the determination of the freezing point as a definite and constant characteristic of genuine milk.

VI. THE OSMOTIC PRESSURE OF MILK AND THE RATIO OF LACTOSE TO CHLORINE.

The question of the exact relationship between the osmotic pressure of blood serum and that of milk is a purely physiological one, and the available data are insufficient to enable any definite conclusions to be drawn. Winter maintained that the osmotic pressure of the milk is directly controlled by that of the blood serum, and that the two are invariably identical, but he did not adduce any conclusive evidence in support of his views. The only experiments carried out simultaneously upon the blood serum and the milk of particular animals are those of Koepe, who found that the freezing points of the two liquids corresponded to a remarkable degree. Nagelschmidt and Strauss both found that if the molecular concentration of the blood is artificially increased, a corresponding increase takes place in that of the milk. It would thus appear probable that the osmotic pressure of the milk bears a direct relationship to that of the blood serum, and if this is the case the observed variations in the freezing point may be supposed to be the result of corresponding variations in the molecular concentration of the blood serum. Whether this is the case or not, the fact that such relatively small variations are observed in the freezing point of milk, even in the case of samples which are more or less abnormal, suggests certain considerations which are of interest from a purely analytical point of view.

The fat in milk has been shown (page 23) to be without influence upon the freezing point. The effect of proteins in solution is so small as to be almost negligible, in fact it is extremely doubtful whether pure proteins, absolutely free from adsorbed salts, exert any influence at all upon the freezing point. The only active substances, therefore, are lactose and soluble salts, together with the comparatively small amount of soluble nitrogenous and other substances which have been shown to exist in milk. A milk which is deficient in lactose may be expected to contain a correspondingly high proportion of soluble salts, and *vice versa*. The soluble salts are largely composed of the chlorides of the alkali metals, and Mathieu and Ferré have recently endeavoured to obtain, from a consideration of the amounts of chlorine and lactose in the milk, a "simplified molecular concentration constant" which will serve as a basis for the calculation of added water.

According to their results, if A represents the number of grammes of hydrated lactose, and B the number of grammes of chlorine (calculated as sodium chloride) per litre of milk, then the expression $A + (B \times 11.9)$, when subjected to a correction for the volume occupied by the proteins and fat in the sample, is approximately a constant and rarely falls below 74 or rises above 79 for genuine milks. In the following table are given the percentages by weight of anhydrous lactose and of chlorine in all the samples on pages 14 to 17, in which chlorine was

estimated,* together with the Mathieu and Ferré figures. The figures are arranged in descending values of lactose.

Sample No.	Mixed Milks.			Sample No.	Milk from Single Cows.		
	Lactose, per cent.	Chlorine, per cent.	Mathieu and Ferré figure.		Lactose, per cent.	Chlorine, per cent.	Mathieu and Ferré figure.
136	5.00	0.082	75.1	106	5.15	0.081	77.4
125	4.99	0.085	76.2	107	5.12	0.080	76.5
140	4.99	0.089	77.3	59	5.08	0.066	73.7
138	4.91	0.082	74.6	64	5.05	0.070	73.1
124	4.88	0.096	76.6	56	5.00	0.071	73.5
135	4.87	0.078	73.6	55	5.00	0.078	75.2
114	4.85	0.085	75.6	58	4.97	0.064	72.1
137	4.81	0.096	76.9	62	4.96	0.057	71.2
126	4.80	0.089	74.6	65	4.94	0.096	76.5
117	4.79	0.085	74.0	110	4.88	0.092	76.4
127	4.78	0.089	75.6	60	4.83	0.064	70.3
116	4.75	0.087	73.4	66	4.77	0.075	71.3
121	4.77	0.088	74.0	61	4.54	0.099	73.2
141	4.72	0.087	73.1	63	4.48	0.071	70.0
113	4.70	0.092	74.2	57	4.48	0.106	74.3
129	4.70	0.103	76.3	108	4.44	0.142	83.0
112	4.65	0.103	76.3	109	3.41	0.188	80.6
132	4.65	0.085	72.7	105	1.80	0.236	78.6
119	4.63	0.103	75.9	111	0.71	0.337	80.8
122	4.63	0.096	74.8				
118	4.61	0.106	76.4				
120	4.60	0.085	72.0				
123	4.60	0.117	78.1				
128	4.57	0.101	75.2				
115	4.55	0.110	75.9				
133	4.55	0.092	72.7				
130	4.50	0.096	73.0				
131	4.50	0.106	74.6				
134	4.29	0.106	72.4				
139	3.81	0.075	60.4				

Samples 105, 108, 109, 111 and 139 were of more or less abnormal character, as will be seen from reference to the analyses on pages 14 to 17. If these samples are eliminated the average values for the Mathieu and Ferré figure on 44 samples is 74.45, the highest being 78.1 and the lowest 70.0, and the per cent. variation from the average $+4.9\%$
 -6.0% .

Out of 44 samples, 17 fall below the value of 74.0, which is given by Mathieu and Ferré as the lower limit for genuine milk. The

* Chlorine was estimated in the ash obtained by ignition in a muffle, at as low a temperature as possible. Mathieu and Ferré estimate chlorine by Volhard's method in the serum produced by means of acetic acid and sodium metaphosphate. A comparison of the following results obtained by both methods shows that no loss of chlorides takes place if the ashing of the milk is carefully carried out in a muffle at a barely perceptible red heat:—

			Chlorine % by analysis of ash.		Chlorine % by Volhard's titration of sodium metaphosphate serum.
Sample A	...	...	0.108	...	0.112
" B	...	...	0.112	...	0.111
" C	...	...	0.105	...	0.109
" D	...	...	0.114	...	0.114
" E	...	...	0.099	...	0.100
" F	...	...	0.115	...	0.114

percentage variation from the average for non-fatty solids and lactose in the 44 samples is $\begin{smallmatrix} +7.8\% \\ -7.3\% \end{smallmatrix}$ and $\begin{smallmatrix} +8.0\% \\ -10.0\% \end{smallmatrix}$ respectively, so that the Mathieu and Ferré figure does not appear to be very much more of a constant than non-fatty solids or lactose. The values obtained do not vary directly as the freezing-point depressions, and a high value for Δ does not necessarily correspond to a high lactose-chlorine figure, or *vice versâ*, although a very rough degree of proportionality may be observed if the two sets of figures are plotted out on squared paper. It is possible that from a consideration of the two values together a more accurate opinion could be arrived at than from the freezing point alone, as to the quantity of extraneous water present.

The figure, as given by Mathieu and Ferré, is based upon the amount of hydrated lactose per litre, and as in this country analytical results are usually expressed in per cent. by weight of anhydrous lactose, the calculation involved, complicated as it is by the necessary corrections for the volume occupied by proteins and fat, is somewhat laborious.

Another paper dealing with this subject has recently been published by Jackson and Rothera. They show that within certain limits the electrical conductivity of milk varies inversely as the lactose. The soluble salts of milk are the only constituents which affect the conductivity to any great extent, lactose, proteins and fat being practically without influence, so that the observations of Jackson and Rothera confirm to some extent the work of Mathieu and Ferré.

VII. CONCLUSIONS.

The average freezing point of 141 samples of genuine milk was found to be -0.5345° , the values found ranging from -0.558° to -0.514° . The values given have been subjected to all the necessary corrections, and are probably accurate to about $\pm 0.002^{\circ}$.

The freezing point appears to be the most constant of any of the properties exhibited by genuine milk. Although unaffected by the removal of fat from, or the addition of separated milk to genuine milk, it is raised by the addition of water to the milk. The method may, in certain circumstances, be applied with advantage, as a confirmatory test, to the detection of added water and to the approximate estimation of the amount present. Owing, however, to the experimental difficulties involved in obtaining reliable results, it is somewhat doubtful whether the method is capable of general application for purposes of milk control.

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November, 1914.

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